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A simple approach to examine early oral microbial biofilm formation and the effects of treatments

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Background/aims: A simple *in vivo* approach to examine early dental plaque formation in the human mouth and to determine the effects of common dietary and oral hygiene procedures on biofilm formation is reported.
Methods: A custom designed device that fits securely behind the teeth of the mandibular arch provides a surface for microbial colonization. This device is prepared with denture acrylic and can be repeatedly used by the subject, exposing a large and constant surface area for microbial accumulation.
Results: Large numbers of oral bacteria colonized the device by 2 h; these increased significantly by 4 h ($P < 0.05$). Bacterial colonization increased significantly after rinsing with a sucrose solution ($P < 0.05$) but remained unaffected after rinsing with water, a commercially available fluoride mouthrinse without antimicrobial agents, or brushing with a fluoride dentifrice ($P > 0.05$). Rinsing with mouthrinses formulated with chlorhexidine, cetylpyridinium chloride or triclosan/copolymer significantly inhibited colonization ($P < 0.05$). A dose-dependent inhibition was noted with chlorhexidine rinses ($P < 0.05$). Brushing with a triclosan/copolymer dentifrice significantly inhibited microbial colonization compared with a control ($P < 0.05$).
Conclusion: This simple approach was useful for examining the effects of common dietary and oral hygiene procedures. Significant biofilm inhibitory effects were noted with formulations that demonstrated efficacy in previous clinical studies.

Key words: *Actinomyces viscosus*; biofilm; chlorhexidine; clinical tests; dental plaque; denture acrylic; mouthrinse; *Streptococcus mutans*; *Streptococcus sanguis*; sucrose; triclosan

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Microbial biofilms are found in many environments. They comprise accumulations of organisms that collectively demonstrate unique properties such as resistance to antimicrobial agents (7). In the human mouth, the unrestricted accumulation of microorganisms on the surfaces of exposed teeth results in dental plaque, a complex microbial biofilm (22). The relationship between dental plaque and oral conditions have been well described, and current dental practices emphasize routine dental plaque control to maintain oral health (22).

The rapid colonization of exposed tooth surfaces by oral bacteria to form dental plaque has been extensively investigated (14). Bacterial colonization of various implant materials reveal that the percentage of different bacterial species accumulating on implant surfaces is comparable to that in supragingival plaque on teeth (9, 21). The utility of removable devices placed in the human mouth to collect dental plaque (27), to examine dentifrice abrasivity (6), fluoride deposition, and enamel remineralization (4) analyze dental plaque, including its microbiology (2, 14, 21, 27), biochemistry (20) and the effects of oral care formulations (3), have been described. Many of these models are cumbersome and require trained dental professionals to conduct the process of sample collection and analysis, and they are, therefore, not useful for routine efforts.

Models ranging from laboratory to *in situ* methods are required to develop and validate treatments for a range of oral conditions, i.e. caries, periodontal disease (18). This report describes a convenient approach to examine early microbial

colonizers in the human mouth, determining the bacterial colonization of a previously clean surface placed in the mouth of human volunteers. The surface for microbial colonization is referred to as a mandibular device or butterfly insert, which is prepared for each volunteer individually with denture acrylic. This reusable device fits behind the mandibular arch of the front teeth, has a large surface area for microbial accumulation, and can be conveniently placed by the study volunteers themselves. At regular time intervals, the effect of specific treatments on the microflora accumulating on the device was determined. The aims of this investigation were to utilize the butterfly device to explore microbial colonization *in vivo* over time and to examine the effects of common oral hygiene procedures on dental plaque formation. Clinically tested oral care formulations containing antiplaque and antimicrobial agents applied by either rinsing or brushing under normal use conditions were examined.

Material and methods

Bacteria, media, and chemicals

Bacterial strains (*Actinomyces viscosus* ATCC 43146, *Streptococcus sanguis* ATCC 10558 and *Streptococcus mutans* ATCC 27351) were obtained from the American Type Culture Collection (Manassas, VA) and maintained in accordance with recommended procedures (16). All bacteriological media were obtained from Becton-Dickinson Co. (Sparks, MD) and prepared according to manufacturer's recommendations. Pre-made agar media for the various studies included Trypticase Soy agar with 5% Sheep Blood (blood agar) and Mitis-Salivarius agar (MS). Chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise indicated. Phosphate-buffered saline (PBS) was obtained from Life Technologies, Gibco-BRL (Grand Island, NY).

Formulations tested

The treatments for the clinical tests included potable water, a commercially available mouthrinse with fluoride and no antibacterial agents (referred to a placebo rinse henceforth), and a sterile aqueous solution of 10% sucrose. Mouthrinse formulations for these clinical studies included commercially available rinses with 0.12% chlorhexidine gluconate (CHX), 0.05% cetylpyridinium chloride (CPC) and a 0.03% triclosan/copolymer rinse. An additional mouthrinse with 0.06% CHX was prepared for tests with all other components identical to the 0.12% CHX rinse. The commercially available dentifrices for these studies included Colgate Total™ (Colgate-Palmolive Company, New York, NY) a dentifrice with 0.3% triclosan and a copolymer of polyvinyl-methyl ether and maleic acid (referred to henceforth as triclosan/copolymer), and a fluoride dentifrice (referred to as control paste).

Preparation of the butterfly device

A custom made device, referred to as a butterfly device because of its shape, was prepared from an impression of the mandible of subjects selected for study. The impression records the negative oral and dental anatomic structures of the subject; a plaster model is then made from this impression, reflecting the positive oral and dental anatomy. Denture acrylic, which is routinely used by dental professionals for dentures, was used to construct the device from the subject's dental model. Subjects visited the dental clinic to ensure a comfortable fit of their completed device. The device is retained in the mouth by the undercut of the embrasure of the natural dentition and put in place by the subjects themselves after suitable instruction. This custom made device (Fig. 1a) fits behind the teeth and was sanded to a uniform roughness (Fig. 1b). Throughout the study, subjects used their own devices, which were thoroughly brushed (without dentifrice), and washed before and after each study. Typically, the device was cleaned with Lysol TM and 70% ethyl alcohol and rinsed with an excess of sterile deionized water and stored in sterile disposable containers with sterilized deionized water and labeled with the subject's name.

Viability of laboratory bacterial cultures and human saliva samples

The microorganisms adhering to the device in the clinical studies was detached at the conclusion of the study by incubation in 0.25% trypsin prior to microbial assessment. Several experiments examined the effects of trypsin on the viability of laboratory cultures of bacteria that are early microbial colonizers of dental plaque. *A. viscosus* ATCC 43146, *S. sanguis* ATCC 10558 and *S. mutans* ATCC 27351 were routinely grown in trypticase soy broth at 37°C under static conditions in accordance with standard procedures

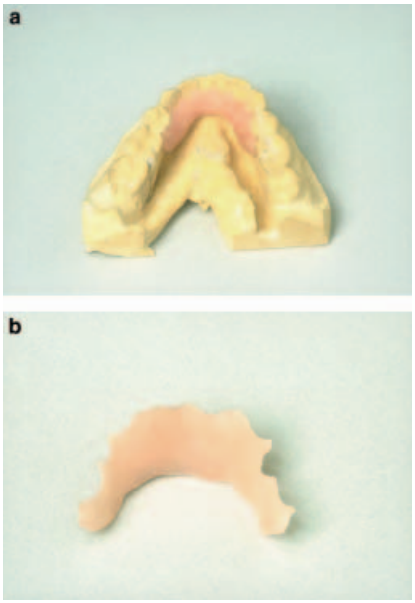

Fig. 1. a) Position of the mandibular insert in the mandible. b) A photograph of the mandibular device provided to subjects for the clinical trials.

(12, 16). The cultures were centrifuged at 7,800 *g* for 10 min at 4°C to harvest the bacteria. The bacterial pellet was resuspended in PBS with 0.25% trypsin and 0.5 mg/ml of L-cysteine-HCl (henceforth referred to as 0.25% trypsin) to an optical density of 0.4 at 610 nm. The viability of the bacterial culture in 0.25% trypsin initially and after 30 min and 60 min of incubation in a shaking water bath at 37°C was determined by plating dilutions in duplicate on appropriate agar media. Plates were incubated as per standard procedures (16, 23) and bacterial colonies enumerated and expressed as $\log_{10}$ colony-forming units (CFU)/ml for viability determinations.

Saliva is probably the principal source of the microorganisms that colonize the device; therefore, the effects of 0.25% trypsin were examined in a study conducted with saliva samples from 22 subjects. For this study, subjects rinsed their mouth with 10 ml of sterile water for 10 s to provide a sample of their oral bacteria prior to oral hygiene procedures. These samples were incubated with trypsin (to obtain a final concentration of 0.25% trypsin) in a shaking water bath at 37°C for 45 min. The numbers of bacteria were determined before and after the trypsin step by plating dilutions on blood agar and bacterial enumeration as described previously (16, 23).

Volunteers for clinical study

Adult volunteers between the age of 18–65 years and in good oral and medical health were recruited for the studies. Subjects were informed of the study procedures and were included based on their ability to comply with specified guidelines. Selected subjects were provided with a commercially available fluoride dentifrice for 1 week of use before the study started; all other oral hygiene formulations, including chewing gum, lozenges, etc., were discontinued for the duration of the study. In addition, subjects currently on prescription medications or who had only discontinued use of such medications in the past 2 months were excluded. Study protocols were approved and trials conducted in accordance with widely accepted clinical practices after obtaining the informed consent of volunteers.

Clinical procedures with the butterfly device

All studies were cross-over in design, with the participants blinded to the treatments, and were conducted after the subjects completed a 1-week washout period with a commercial fluoride dentifrice. After the washout phase, subjects arrived at the dental clinic after breakfast and prior to undertaking oral hygiene procedures. Their butterfly device was soaked in a sterile plastic disposable tube containing an excess (40 ml) of 70% ethanol and shaken by hand for 2 min. The ethanol was decanted and the device transferred to a fresh sterile plastic disposable tube containing 40 ml of sterile deionized water and shaken for 2 min. This step was repeated to wash the device twice with sterile water. Volunteers washed their hands with soap and water and swabbed their palms with 70% ethanol prior to placing the device in their mouth. Next, subjects rinsed with the assigned treatment (as described below) and refrained from food or drink for the next 2–4 h depending on the study design but were allowed to drink water if required. Volunteers returned to the dental clinic at their appointed time and decontaminated their hands with 70% ethanol. The device was removed and gently rinsed with 6 ml of sterile deionized water to remove debris and loosely adhering saliva. The device was completely immersed in 0.25% trypsin (15 ml) and incubated at 37°C for 45 min prior to microbial estimation as described below. This concluded day 1 of the test. The second part of the test was held 2 days later, and was referred to as day 3 of the study. With the day 1 and day 3 design, the baseline microbial numbers from subjects could be estimated each week on day 1 and compared to the number of bacteria following the use of antimicrobial formulations (mouthrinse or dentifrice) on day 3.

Effects of treatments on microbial flora colonizing the device

Initial studies examined biofilm formation on the device over time. With a group of 13 subjects, the effects of rinsing with 15 ml potable water on biofilm formation at 2 h and 4 h were examined with the treatments (2 h or 4 h of biofilm formation in the mouth) randomized between day 1 and day 3 of the trial. In a group of 12 subjects, the effects of rinsing with an aqueous solution of 10% sucrose (15 ml for 45 s) were compared to rinsing with potable water (15 ml for 45 s) on biofilm formation. The treatments were randomized between day 1 and day 3 with effects examined 4 h post-rinsing.

A clinical study of mouthrinses compared the effects of rinsing with potable water and a commercially available fluoride mouthrinse in 15 subjects. For this test, the treatments were randomized on days 1 and 3; biofilm formation was examined 4 h post-treatment. To examine the efficacy of mouthrinses with antimicrobial agents (triclosan/copolymer, CPC, CHX rinses at 0.12% or 0.06%), the placebo rinse was provided on day 1 and the antimicrobial rinse on day 3. Subjects rinsed for 45 s with 15 ml of each rinse, and abstained from any food or oral hygiene procedures for the next 4 h. Another study compared the effects of brushing with the control paste on day 1 (with 1.5 g of paste for 45 s) to rinsing with 0.12% CHX (for 45 s) on day 3 on biofilm formation at 4 h.

Initial studies with dentifrices examined the effect of brushing with the control fluoride dentifrice on days 1 and 3. This served as a control study to determine the effects of brushing on microbial colonization of the device on day 1 and day 3. To test the effects of dentifrices with antiplaque agents, eight subjects were given the control paste on day 1 to obtain a baseline level of the oral microflora; the antiplaque formulation (triclosan/copolymer) was then tested on day 3. With all dentifrices, subjects brushed with 1.5 g of dentifrice dispensed on a new soft bristled brush for 45 s and swished the foam around in the mouth for 15 s. The foam and paste were expectorated and subjects rinsed for 15 s with 15 ml tap water and refrained from food or drink for the next 4 h. After 4 h, the device was removed from the subject's mouth and the number of bacteria on the device enumerated as described below. This concluded day 1 of the test. Volunteers were scheduled for the second part of the study 2 days later (day 3).

Microbiological procedures for the butterfly device

All devices were incubated in 0.25% trypsin (15 ml) for 45 min in a shaking water bath at 37°C. Following the trypsin step, the contents of the tube were vortexed thoroughly and dilutions prepared in PBS. These dilutions were plated in duplicate on blood agar to quantify the number of all cultivable oral bacteria. In some experiments, dilutions were also plated onto MS agar to enumerate oral streptococci. All bacteriological media were incubated under standard conditions as described previously (16, 23) for bacterial enumeration.

Statistical analysis

Bacterial counts (CFU/ml) from duplicate plates were transformed to $\log_{10}$ and averaged for statistical analysis. Comparisons between each treatment group were made using *t*-test analysis with the Microsoft EXCEL program with significant effects determined at $P < 0.05$.

Results

In vitro viability of bacterial cultures and saliva in PBS with 0.25% trypsin

The bacteria adhering to the insert in the clinical studies were detached by incubation in a solution of trypsin prior to quantification by plating dilutions on bacteriological media. *In vitro* experiments examined the viability of bacterial cultures of *A. viscosus, S. sanguis and S. mutans* suspended in PBS containing 0.25% trypsin and 0.5 mg/ml of L-cysteine-HCl. These bacteria are members of the early dental plaque (14, 22, 23) and were chosen because they represented the predominant members colonizing the device. The viability of each bacterial culture was determined initially and after 30 and 60 min of incubation in a shaking water bath set at 37°C. Duplicate *in vitro* experiments indicated no loss of viability among the three laboratory strains after up to 60 min of incubation in 0.25% trypsin (data not shown).

Saliva probably serves as the primary source of bacteria colonizing the device; therefore, an experiment was carried out to examine the viability of oral bacteria in 0.25% trypsin in saliva samples obtained from 22 subjects. No statistical differences were found in the viable bacteria following incubation in trypsin for 45 min at 37°C (Table 1).

Number of oral bacteria resident on the insert over time

The number of cultivable oral microflora and members of oral streptococci resident on the device were examined following either 2 or 4 h of placement in the subject's mouth. For this cross-over design study, 13 subjects were randomly assigned the treatment period after rinsing with potable water. The results (Fig. 2) indicate a significant increase in the number of cultivable oral flora on the insert from 2 h to 4 h ($P < 0.05$). The number of oral streptococci on the insert also increased from 2 h to 4 h, but this increase was insignificant ($P > 0.05$). The 4-h period was used in further clinical studies with the insert to allow the greatest accumulation of oral bacteria.

Effect of rinsing with sucrose on bacteria colonizing insert

Sucrose, a common food ingredient, is utilized by a variety of oral bacteria to produce glucans (14, 15, 22). Therefore, a study examined the number of bacteria on the insert 4 h after rinsing with 10% sucrose in comparison with potable water in a group of 12 subjects. The sucrose rinse resulted in a significant increase in the number of oral biofilm ($P < 0.05$) (Fig. 3). The number of oral streptococci also increased following the sucrose rinse; however, this increase was not statistically significant ($P > 0.05$).

Effects of common oral hygiene procedures on oral biofilm formation

Preliminary studies compared the effects of brushing with a commercial fluoride dentifrice on day 1 and day 3 of the study to examine the effects on biofilm formation and the influence of brushing procedures. The average recovery of biofilm bacteria from this cross-over design study with seven subjects were 5.33 ± 0.53 log CFU/ml and 5.52 ± 0.51 log CFU/ml, on day 1 and day 3, respectively. These results were not statistically significant ($P > 0.1$).

Table 1. Viability of salivary microflora from 22 subjects in 0.25% trypsin

Incubation time (exposure to 0.25% trypsin)	Average ± standard deviation of cultivable oral microflora (Log CFU/ml)
0 min	6.64 ± 0.36
45 min	6.77 ± 0.41

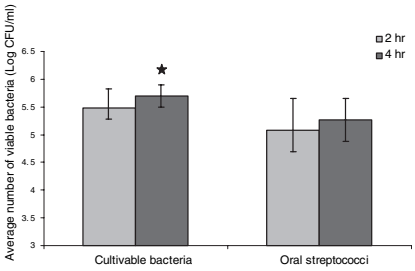

Fig. 2. Biofilm bacteria on the mandibular insert over time. Results indicate average ± standard deviation (Log CFU/ml) of bacteria recovered from 13 subjects in the clinical trial (***** indicates statistically significant differences in cultivable bacteria between 2 and 4 h ($P < 0.05$)).

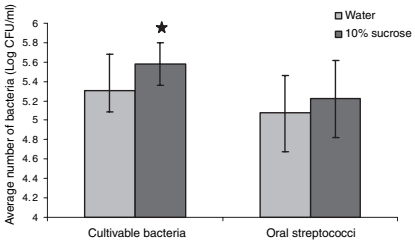

Fig. 3. The effect of rinsing with 10% sucrose on biofilm bacteria. Results indicate average ± standard deviation (Log CFU/ml) of bacteria recovered from 12 subjects in the clinical trial (***** indicates statistically significant increase in cultivable bacteria after rinsing with the 10% sucrose rinse ($P < 0.05$)).

A common oral hygiene procedure is rinsing the mouth with potable water. Therefore, a study compared the effects of rinsing with potable water and a placebo mouthrinse on the numbers of oral biofilm bacteria. In this cross-over design study with 15 subjects, 5.65 ± 0.35 log CFU/ml and 5.56 ± 0.35 log CFU/ml of cultivable biofilm bacteria were recovered after the use of potable water and placebo mouthrinse, respectively. The numbers of oral streptococci recovered were 5.27 ± 0.52 log CFU/ml and 5.24 ± 0.42 log CFU/ml following the use of potable water and placebo mouthrinse, respectively. No statistically significant differences in the numbers of cultivable bacteria or oral streptococci were noted between potable water and the placebo mouthrinse ($P > 0.1$).

Effects of antimicrobial rinses on oral biofilms

Two separate cross-over design studies with 14 subjects compared the effects at 4 h post-use of a commercially available fluoride mouthrinse with no antimicrobial agents to the use of commercial mouthrinses formulated with 0.03% triclosan/copolymer or 0.05% CPC. Results indicate a statistically significant decrease in cultivable oral bacteria when triclosan/copolymer and the CPC rinse were compared with the placebo (57% and 53%, respectively) (Fig. 4). A 64% and 40% decrease in the oral streptococci were noted with the triclosan/copolymer and the CPC rinse, respectively, at 4 h compared to the placebo rinse ($P < 0.05$).

Chlorhexidine is a potent antimicrobial agent commonly formulated in mouthrinses. The antibacterial effects of a 0.12% CHX rinse were compared to brushing with a commercial fluoride dentifrice in a study with nine subjects. The average number of oral bacteria from subjects at 4 h following brushing with the fluoride dentifrice and rinsing with the CHX rinse were 5.47 ± 0.52 log CFU/ml and 4.01 ± 1.6 log CFU/ml, respectively, representing a statistically significant 1.46 log CFU/ml decrease using the CHX rinse ($P < 0.05$).

The dose-dependent effects of CHX rinses formulated with 0.06% CHX and 0.12% CHX were determined with nine subjects. For these studies, the CHX rinses were randomly assigned to subjects on day 3 with a 5-day washout phase between the 2-week study periods. The results from these studies (Fig. 5) demonstrate the significant effects of each CHX rinse on both the cultivable bacteria and oral streptococci vs. the placebo rinse ($P < 0.05$). Additional analysis indicates the significant dose-dependent effects of the CHX rinses on cultivable oral bacteria (1.61 and 1.56 log CFU/ml decrease for the 0.12% CHX and 0.06% CHX rinse, respectively) and oral streptococci (2.23 and 1.36 log CFU/ml decrease, respectively) ($P < 0.05$).

Effects of brushing with dentifrices formulated with antimicrobial agents on oral biofilms

A study compared the effects of brushing with the triclosan/copolymer to a commercially available fluoride dentifrice. In this cross-over design study, eight subjects brushed once with microbial accumulation on the device

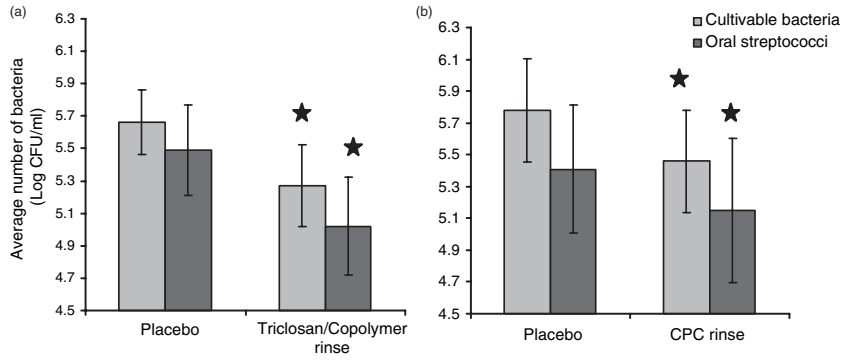

Fig. 4. Inhibition of oral biofilm formation by a 0.03% triclosan/copolymer rinse (A) and a 0.05% cetylpyridinium chloride rinse (B) in comparison to a placebo mouthrinse. Results indicate average ± standard deviation (Log CFU/ml) of bacteria recovered from 14 subjects in the clinical trials following each treatment (★ indicates significantly lower recovery of cultivable bacteria and oral streptococci after rinsing with the triclosan/copolymer rinse or CPC rinse vs. placebo ($P < 0.05$)).

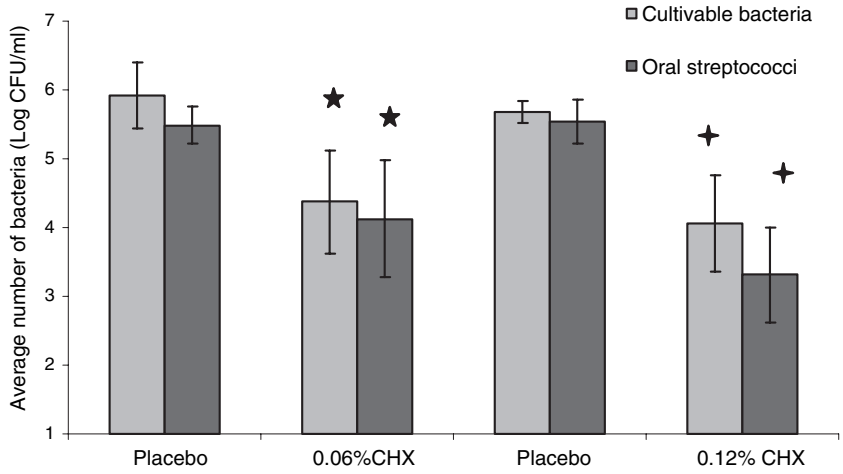

Fig. 5. The dose-dependent efficacy of CHX mouthrinses on oral biofilm formation. Results indicate average ± standard deviation (Log CFU/ml) of bacteria from nine subjects in the clinical trial (★ or ✦ indicates statistically significant differences in cultivable bacteria and oral streptococci after the use of the respective CHX rinse vs. placebo rinse ($P < 0.05$); + also indicates statistically significant differences with the 0.12% CHX vs. the 0.06% CHX rinse for cultivable bacteria and oral streptococci ($P < 0.05$)).

determined at 4 h postbrushing. Brushing with the triclosan/copolymer dentifrice resulted in fewer bacteria on the device than the control fluoride dentifrice. On average, brushing with triclosan/copolymer dentifrice resulted in a statistically significant 44% reduction of oral bacteria vs. the control dentifrice ($P < 0.05$) (data not shown).

Discussion

Dental biofilm (plaque) formation *in vivo* is characterized by a rapid acquisition of the salivary pellicle rich in proteins and glycoproteins followed within a few minutes by colonizing oral bacteria on surfaces, including plastic and acrylic (10, 14, 21, 23). Research indicates that colonization by oral bacteria increases over time. The initial dental plaque (up to 8 h) comprises predominantly oral streptococci with sizable proportions of *Actinomyces* sp. and representatives of other microbial genera (14, 22). The predominance of cocci in initial plaque at 2 h, with significant increases by 4 h, has been corroborated by scanning electron microscopy (21). Further, the percentages of different microorganisms in dental plaque formed at 4 h on acrylic surfaces *in vivo* are similar to those in supragingival plaque (9, 10). Relationships in the kinetics of dental plaque accumulation on various implant surfaces in relation to enamel analyzed by clinical plaque indices and elemental microanalyses of plaque are known (1). The appropriateness of using dental acrylic surfaces to collect and analyze salivary pellicle and dental plaque, is supported by these observations, and led to this investigation. For this study, a convenient device with a large surface area was developed for plaque collection and analysis. The device is designed as a single unit with no removable parts to minimize the steps associated with analysis. As seen with enamel and tooth surfaces, colonization of the device by oral bacteria increased significantly from 2 to 4 h.

Clinical results from *in vivo* models indicate that all materials placed in the human mouth collect microbial biofilms depending on surface roughness and free energy (19). However, surfaces of similar surface roughness have a similar percentage and composition of early microbial colonizers from 10 min to 72 h of oral colonization (10, 13, 21). In the present *in vivo* model, subjects repeatedly used their own devices in the studies. The procedures for the preparation of these devices were standardized to reduce the influences of surface energy and roughness on biofilm formation. Additionally, subjects completed crossover design studies to minimize the influence of host parameters.

Dental plaque was dislodged from the devices using incubation with trypsin. Laboratory investigations demonstrate that several oral bacteria are resistant to treatment with low concentrations of enzymes (8, 28). The viability of common oral bacteria that colonize the oral biofilm at an early stage or in salivary samples that reflect oral bacteria from volunteers were not reduced following trypsin treatment. A large number of diverse oral flora are found in the saliva, as shown in a recent report utilizing DNA–DNA checkerboard analysis (17). A slight increase in viable bacteria was noted following the trypsin step, potentially suggesting a decrease in the microbial clumps.

A significant increase in oral bacteria was observed on the device after rinsing with a sucrose solution and is comparable to earlier results (15). Further, increases in streptococci were noted and indicate the feasibility of characterizing the types of bacteria colonizing the device. The results also suggest the suitability of this model for examining the effect of food ingredients on dental plaque formation. Additionally, the effectiveness of common oral hygiene procedures, i.e. rinsing with potable water or commercial fluoride

mouthrinses on microbial colonization, were investigated.

The clinical studies indicate the significant inhibitory effects of oral care formulations with CHX, triclosan/gantrez and other antiplaque agents on dental plaque (5). With mouthrinses formulated with CPC or triclosan/gantrez, a significant decrease of microbial biofilm formation was noted with the device, similar to previous clinical effects seen on supragingival plaque. To further examine the appropriateness of the device, studies compared a CHX mouthrinse to brushing with a fluoride dentifrice. The CHX rinse significantly inhibited microbial colonization. Clinical studies comparing brushing and rinsing regimens are infrequent in the literature. The current results indicate that this new model can compare different approaches, i.e. brushing to rinsing, for examining the effects of antiplaque agents. Routine brushing with a commercial fluoride dentifrice did not influence oral biofilm formation. On the other hand, brushing with a dentifrice with triclosan/copolymer demonstrated significant inhibitions of bacteria colonizing the device. These results are in agreement with the significant clinical effects of the triclosan/copolymer dentifrice in reducing supragingival dental plaque reported from a number of clinical studies (26). To further examine the utility of the device, the dose-dependent effects of CHX mouthrinses on biofilm inhibition were determined. The control experiment that demonstrated a lack of significant effects after brushing with a fluoride dentifrice over the course of the study suggests that the mechanics of placing the device in the mouth and brushing do not influence microbial accumulation.

In summary, clinical trials indicate the utility of the butterfly device with simple procedures to assess the effects of specific ingredients common in food or oral hygiene procedures and the effects of common antiplaque agents. The results will allow test hierarchies to be designed (18) for agents that may affect early plaque or inhibit microbial adhesion. Additionally, the device can serve as a biofilm collection platform for microbial analysis using molecular (16) and microscopic approaches (2, 9) to characterize microbial community dynamics. The advantages of the butterfly device include the few preparatory efforts needed to collect dental plaque and its application for examining tartar formation and possibly for quantifying the amount of active following the use of specific formulations. The available data also serves as a basis for future studies to monitor specific characteristics (oral biofilm, tartar formation, etc.) at selected periods of a longer clinical trial in order to determine effects over time.

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